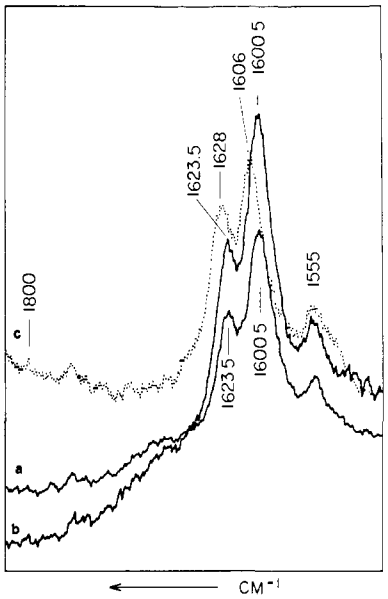

Figure 2. Resonance Raman spectra: (a) Spectrum of a mixture of ester substrate **2** (3.3×10^{-4} M) with CPA_α (4.5×10^{-4} M) at −25 °C (solid line, top). The solvent that had an apparent pH of 7.5 consisted of 50% ethylene glycol, 50% aqueous 0.1 M sodium cacodylate, and 0.5 M NaCl. Recording of the spectrum was started approximately 5 s after mixing, at a point where the reaction of **2** with CPA was in the "slow" phase of the biphasic kinetics seen. The peaks observed for *p*-(dimethylamino)-cinnamic propionic anhydride in CH_3CN occur at 1622 and 1598 cm^{-1} and are both expected to be 1-2 cm^{-1} lower under the solvent conditions of this experiment. The spectra were obtained using a diode array-based multiplex instrument described in ref 17. Approximately 200-mW, 350.7-nm laser excitation with 20-s total acquisition time was employed. Minor features in the 1650-1800-cm^{-1} region are also present in the spectra of the solvent–enzyme mixture alone. (b) Spectrum of ester **2** (3.3×10^{-4} M) added to a mixture of the competitive inhibitor benzylsuccinate (1.5×10^{-3} M racemate) with CPA_α (4.5×10^{-4} M) at −25 °C (solid line, bottom). The conditions employed were similar to those described above. (c) Spectrum of the solution resulting from allowing a mixture of ester **2** (3.3×10^{-4} M) with CPA_α (4.5×10^{-4} M), to be incubated at −25 °C and then maintained at 6 °C for 5 min (dotted line). The solvent conditions corresponded to those employed in parts a and b above. Under these conditions turnover had occurred.

is reminiscent of the hydrolysis of 3,5-dinitroaspirin where an equilibrium with an anhydride intermediate favoring the starting material is thought to occur but anhydride breakdown is rate limiting.[16] In any event, what does emerge clearly from the present study is that any interpretation of physical data, based on the premise that the mixed anhydride accumulates in mixtures of esters such as **1** or **2** with CPA at low temperatures in aqueous–organic solvents, is fraught with danger.

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(15) The observation that L-β-phenyllactate acts as a competitive inhibitor in the CPA-catalyzed hydrolysis of several esters has been cited in kinetic arguments in ref 14 that the complexes related to ES′ of eq 1 cannot be acyl-enzymes. This argument is based upon the assumption that L-β-phenyllactate must be released to solution if it is formed by acylation of CPA with a corresponding ester. However, if trapping of L-phenyllactate in the hydrophobic binding pocket of the enzyme occurs due to steric hindrance resulting from acylation of CPA by the acyl group of the acyl-L-β-phenyllactate, as is suggested by a molecular model of the enzyme, then release of L-β-phenyllactate to solution would take place only as the acyl-enzyme decomposes. In this case the observation of competitive inhibition by L-β-phenyllactate would not be inconsistent with the intermediacy of an anhydride species.

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(19) Present mailing address of E.T.K.: Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, NY 10021.

Selective DEPT Pulse Sequence. A Rapid One-Dimensional Experiment for the Simultaneous Determination of Carbon–Proton Chemical-Shift Correlations and CH_n Multiplicities

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Multipulse techniques are finding increasing use for facilitating the structure elucidation of organic molecules by NMR spectroscopy. Much structural information can be gleaned by the generation of $^{13}C,^1H$ chemical-shift correlation maps, and a variety of two-dimensional NMR procedures are available for this purpose.[1] A number of one-dimensional editing pulse sequences have been developed recently for the determination of CH_n multiplicities with the DEPT pulse sequence being probably the procedure of choice.[2] Ernst and co-workers have developed a two-dimensional version of DEPT that generates simultaneously the required chemical-shift correlation *and* edits the two-dimensional contour map into CH, CH_2, and CH_3 subregions.[3] In this paper, we describe a selective version of DEPT that can be utilized as a one-dimensional NMR procedure for the generation of $^{13}C,^1H$ chemical-shift correlations (to an accuracy of 0.05 ppm in the ^1H spectrum) and, if desired, to acquire information concerning CH_n multiplicities.

The selective DEPT pulse sequence is as follows:

$$\pi(H_a)(\pi/2)[H,\pm x](\pi/2) \times [C,y]-1/(2J)-\pi[C,\pm x]\theta[H,y]-1/(2J)-(\pi/2)[C,\pm y] \text{ acquire } ^{13}C \text{ with receiver phase cycling, decouple proton (A)}$$

$\pi(H_a)$ is a selective pulse applied to one-half of the $^{13}C,^1H$ doublet in the ^1H spectrum. The chemical-shift correlation is generated because of the selective nature of the soft $\pi(H_a)$ pulse. The remaining pulses are hard pulses. The soft $\pi(H_a)$ pulse causes a selective population inversion; double-quantum coherence is now generated for CH_n spin systems following the hard-pulse combination $(\pi/2)[H,\pm x](\pi/2)[C,y]$. This coherence is allowed to evolve for a time period $(2J)^{-1}$ after which it is converted to observable single-quantum coherence by the $\theta[H,y]$ pulse. These processes have been discussed in detail elsewhere.[3,4] Because of the selective nature of the experiment, no $\pi[H]$ pulses are required. The pulse combination $\pi(H_a)(\pi/2)[H,\pm x]$ is equivalent to the initial pulse segment $(\pi/2)[H,\pm y]-1/2J-$ of the basic DEPT pulse sequence

$$(\pi/2)[H,\pm y]-1/(2J)-\pi[H](\pi/2) \times [C,y]-1/(2J)-\pi[C]\theta[H,x]-1/(2J)(\pi/2)[C,\pm y] \text{ acquire } ^{13}C \text{ with receiver phase cycling, decouple proton (B)}$$

The difference in phase of the θ-pulses between sequences A and B arises as a consequence of $\pi(H_a)$ being applied $J/2$ Hz off resonance.

Pulse seqeunce A can be implemented in a variety of ways dependent upon the structural information sought. Because of the functional dependence of the intensity[4] of the observed ^{13}C signal with θ (sin θ for CH, sin θ cos θ for CH_2, and sin θ $\cos^2$ θ for CH_3 carbon signals) if θ is set equal to $\pi/2$, $^{13}C,^1H$ chem-

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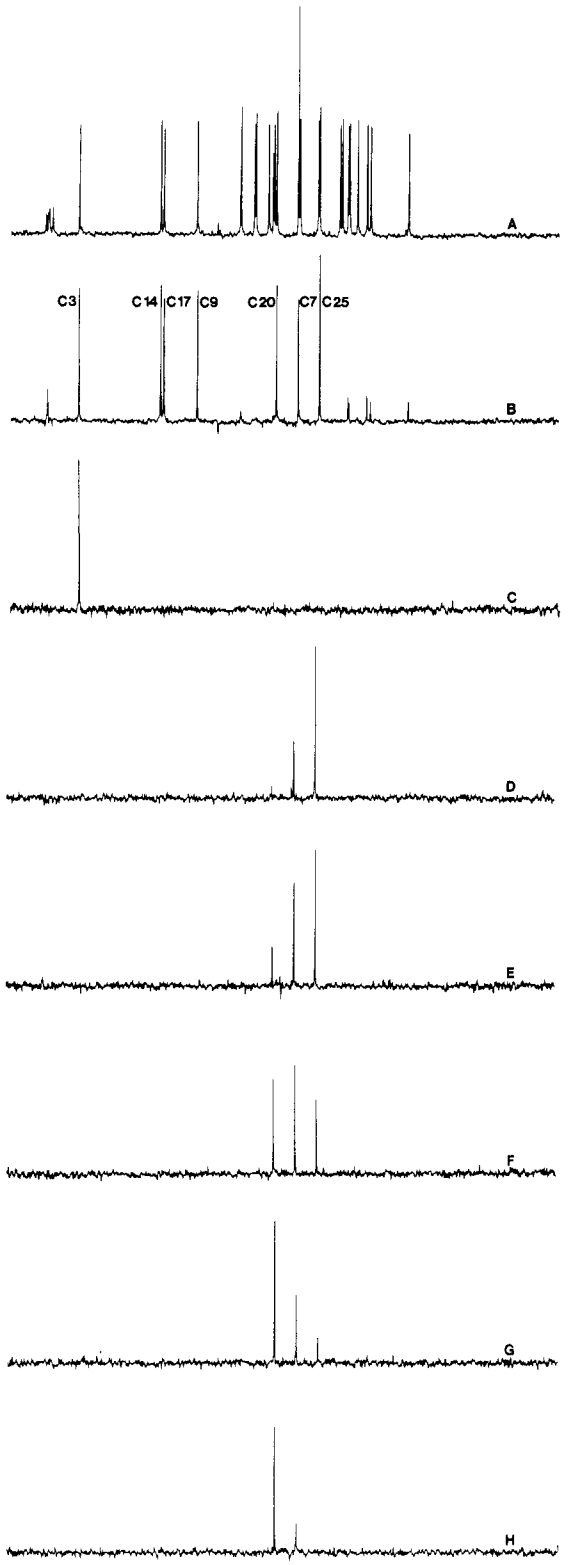

Figure 1. (A) Upfield region of the normal FT NMR spectrum of cholesterol in $CDCl_3$. 32 pulses were averaged by using a recycle time of 5 s; a spectral width of 5000 Hz is displayed. (B) Upfield region of the hard-DEPT spectrum of cholesterol determined with $\theta = \pi/2$ showing the expected seven CH carbon resonances in this region of the spectrum. Note the CH_3 error peaks.[2] (C–H) Selective-DEPT spectra determined with $\theta = \pi/2$ showing the position of maximum enhancement for C3 and the effect on the peak intensities of C7, C20, and C25 as the ^1H frequency is incremented by 10-Hz steps through the ^1H spectrum.

ical-shift correlations are generated for CH-type carbons exclusively. If ν_H is the ^1H resonance frequency, selective enhancements occur at $\nu_H \pm J/2$ where J is the $^{13}C,^1H$ coupling constant. As

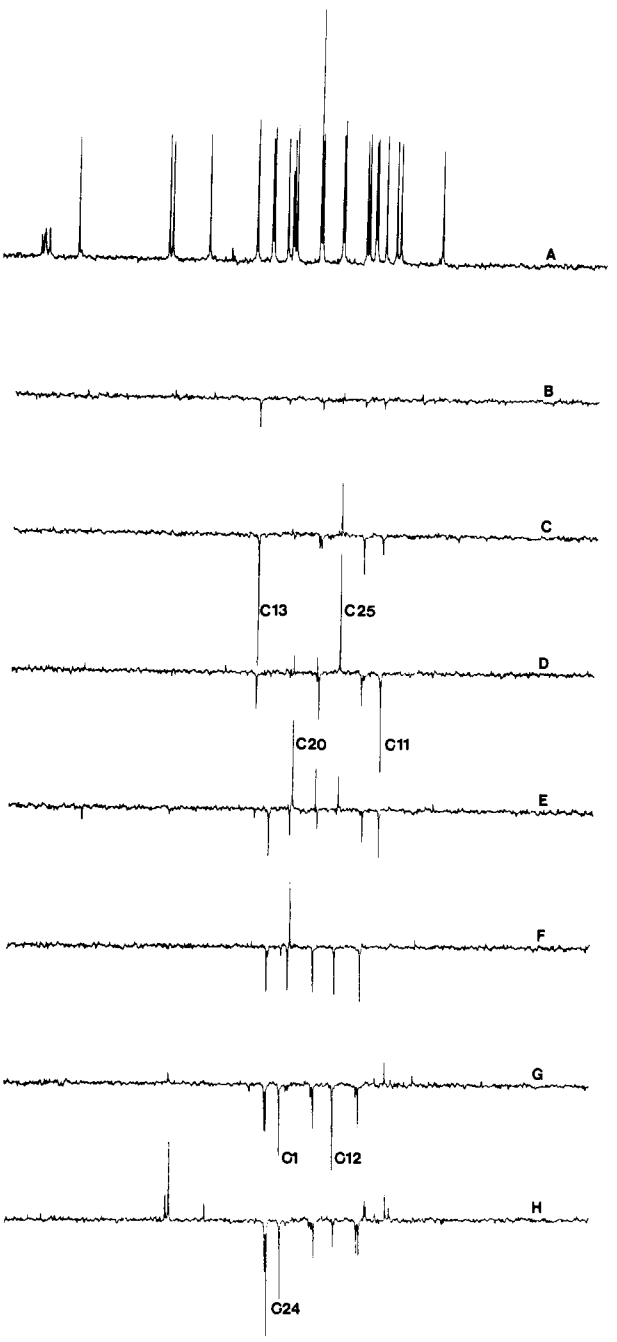

Figure 2. (A) Upfield region of the normal FT NMR spectrum of cholesterol in $CDCl_3$, as in Figure 1. (B–H) Upfield region of the selective-DEPT spectra determined with $\theta = (3\pi/4)$ showing the effect on the intensity of selected peaks as the ^1H frequency is stepped by 20-Hz increments through the ^1H spectrum.

the position of maximum enhancement can be determined to an accuracy of ±5 Hz, this corresponds to an error of ±0.05 ppm at 200 MHz in the determination of the ^1H chemical shift. This degree of accuracy is sufficient for most purposes.

In Figure 1 are shown spectra[5] of cholesterol determined with θ set equal to $\pi/2$. In all cases, the signal determined by exciting the appropriate downfield satellite in the proton spectrum is displayed. Note the excellent suppression of undesired signals; these include all signals resonating in the spectral range displayed arising from the natural ^{13}C magnetization and those CH_2 and

(5) Selective-DEPT ^{13}C spectra were determined at 50.31 MHz on an extensively modified Bruker spectrometer. The sample was approximately 1 M in $CDCl_3$. Pulse times were as follows: $t_{90}(^1H) = 27.5$ μs, $t_{90}(^{13}C) = 21$ μs, soft $t_{180}(^1H) = 30$ ms. The pulse-train recycle time was 5 s, and $(2J)^{-1}$ was set equal to 3.7 ms.

CH_3 signals that would be observed with θ mis-set from a pulse angle of $\pi/2$ radians. As well, note the sensitivity of the position of maximum enhancement to the setting of the ^1H excitation frequency. We readily conclude that the $^{13}C,^1H$ chemical-shift correlations in ppm for aliphatic CH-type carbons are as follows: C3 (72.8, 3.5), C8 (33.1, 1.4), C9 (51.4, 0.8), C14 (58.0, 1.0), C17 (57.4, 1.1), C20 (37.0, 1.4), and C25 (29.2, 1.5). A useful variation for implementing sequence A is as follows: Because of the functional dependence of the signal intensity with θ, if θ is set equal to $(3\pi)/4$, CH and CH_3 signals will appear in phase, while CH_2 signals will appear phase inverted. A subset of chemical-shift correlation spectra for cholesterol determined with θ set equal to $(3\pi)/4$ are shown in Figure 2. It is probable that this variation will be the method of choice for the implementation of sequence A as not only are the chemical-shift correlations generated but a degree of multiplicity determination is achieved as well. Of course the only ambiguity remaining is the differentiation between CH and CH_3 carbon resonances; this is readily resolved if a hard-pulse version of DEPT is used to determine a complete CH subspectrum; that is, θ is set equal to $\pi/2$ in sequence B.

The major advantages of the selective DEPT sequence are as follows: (1) As no second Fourier transformation is required, the technique is faster in terms of data processing time than existing heteronuclear 2D chemical-shift correlation procedures. (2) Because a selective position of the ^1H spectrum can be interrogated, there can be a significant time saving over existing 2D procedures. For example, when NMR is applied for structure determination of a complex natural product, often all that might be required, or indeed useful, is to generate chemical-shift correlations for selected resolved proton resonances. This is a trivial matter using the above procedure and can be carried out by recording only a few ^{13}C spectra. Although other tecniques, such as selective decoupling or DANTE-induced selective population inversion,[6] are available for this purpose, the spectral quality is often much poorer.

Acknowledgment. This research is supported by the Australian Research Grants Scheme.

Registry No. Cholesterol, 57-88-5.

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Synthesis of the Alleged Structure of Senoxydene, the Triquinane Sesquiterpene Derived from *Senecio oxyodontus*

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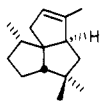
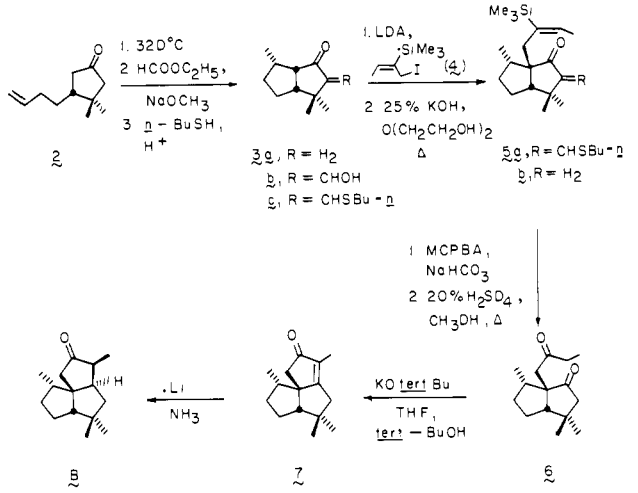
Senoxydene, a sesquiterpene hydrocarbon isolated by Bohlmann and Zdero in 1979 from *Senecio oxyodontus*,[2] was assigned the angular triquinane structure **1** on the basis of detailed spectroscopic



1

Scheme I



analysis. The unusual arrangement of the methyl substituents in **1**, which differentiates it from isocomene, silphinene, and pentalenene, has provided the impetus for biogenetic considerations[3] linking senoxydene to botrydial and quadrone. The current widespread interest in naturally occurring polyquinanes[4] prompts us to report at this time an unambigous, fully stereocontrolled synthesis of compound **1** which demonstrates that senoxydene cannot be constituted as originally proposed.

Careful retrosynthetic analysis of the substitution plan in **1** suggested that elaboration of the lower bicyclo[3.3.0]octane moiety, with proper attention to the stereochemical relationship of the secondary methyl group to the angular proton, might well be pursued first. Adoption of this protocol would require that a new cyclopentane annulation scheme be later implemented in a manner that would set the endocyclic double bond and associated methyl group regiospecifically into position.

With these goals in mind, 4,4-dimethylcyclopentenone was treated with 4-butenylmagnesium bromide in the presence of cuprous bromide–dimethyl sulfide complex to give **2** (65%).[5] When heated in a sealed tube at 320 °C for 80 min, the trisubstituted cyclopentanone smoothly entered into intramolecular ene cyclization[6] to provide epimerically pure **3a** (78%). Because the invariant response of **3a** to a host of bases was to produce the less substituted enolate irrespective of conditions, functionalization of the central α-carbonyl site required preliminary conversion to **3c** (62% overall). Subsequent deprotonation with lithium diisopropylamide in tetrahydrofuran at −30 °C and treatment with (*E*)-1-iodo-2-(trimethylsilyl)-2-butene (**4**)[7] led to **5a**. Although significant levels of strain and steric congestion had to be overcome, the primary nature of the leaving group in **4** and careful optimization of the alkylation conditions (−30 °C, warm to 20 °C during 4 h) consistently delivered **5a** in 45% yield. For the usual reasons,[4] retention of cis stereochemistry in the bicyclooctanone was fully expected (Scheme I).

Following removal of the blocking group (reflux, 48 h, 45%), the vinylsilane functionality was converted quantitatively into the epoxide. Without purification, acid-catalyzed isomerization[8] resulted in conversion to diketone **6** (65%). Base-promoted cyclization furnished enone **7** in 82% yield. The structural assign-

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(1) Author to whom inquiries regarding the X-ray analysis should be directed at the Merck address.

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(5) All new compounds exhibited compatible infrared, proton magnetic resonance, and mass spectroscopic data. In addition, the elemental composition of all key intermediates has been substantiated by combustion analysis. Yields refer to isolated chromatographically homogeneous materials.

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